

## Warm ischaemia is an important pre-analytical variable affecting the analysis of endometrial biospecimens

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**Introduction:** Biobanks hosting patient biospecimens are central to endometrial research, however pre-analytical variables including specimen handling can lead to misleading, not reproducible and clinically untranslatable results. Warm ischaemia is a putative variable that significantly affects downstream analysis, but its effect on endometrial biopsies is yet to be investigated, and further understanding is vital for generating robust data and the harmonisation of biobanking.

**Aims:** To examine the effect of warm ischaemia on the analysis of endometrial cancer (EC) and healthy endometrial samples.

**Method:** Paired healthy (n=5) and malignant (n=22) endometrial samples were taken before and after hysterectomy for comparison of expressional analysis. Methods of immunohistochemistry and RT-qPCR were used to examine the expression of HIF-1 $\alpha$  and genes of interest *VEGFA*, *CA9* and *PGR*.

**Results:** *VEGFA* ( $p=0.036$ ) and *PGR* ( $p=0.035$ ) genes were upregulated in normal endometrial samples exposed to warm ischaemia, however subsequent analysis confirmed *VEGFA* upregulation to be an artefact due to myometrial contamination. Prognostic gene *PGR* expression in normal post-hysterectomy samples negatively correlated with inter-biopsy time, accounting for warm ischaemia time ( $R^2=-0.66$ ,  $p=0.0043$ ). Gene expressional analysis in EC samples and immune-staining of HIF-1 $\alpha$  was unchanged by warm ischaemia.

**Conclusion:** Analysis of the markers examined in EC samples is not significantly altered by warm ischaemia. Myometrial contamination may affect gene expressional analysis.